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Structural diversity in Cumin seeds (*Cuminum cyminum* L.) using SEM and AFM

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Abstract : Seeds of *Cuminum cyminum* L. were collected from two states of India, Gujarat and West Bengal. The ecological conditions of the two states are quite different and the quantity of the crop production is also different. The microstructural analysis of the two strains (each 30 in number) was done by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) after the same period of time following harvesting. Both the high resolution microscopy techniques reveal specific differences at different levels between the two varieties of the seed. Seed structures which have been analysed under high resolution microscope clearly show the difference in development of furrows and ridges. The papillose hairs are quite dissimilar in length and structure as evident from the SEM images. The topographic and phase images of AFM are significantly different in the two varieties of seeds.

Key words : Cumin seed, microstructure, SEM, AFM.

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In India, cumin (*Cuminum cyminum* L. – family Apiaceae) is cultivated mainly in the states of Gujarat, Rajasthan, Uttar Pradesh, Punjab and Tamil Nadu and much less in a few other states. As the seeds are of immense medicinal importance and have a distinctive aromatic flavour, the nutritional value of the seeds has been studied thoroughly [1]. This spice is in great demand in India and abroad and a valuable source of foreign exchange [2]. The microscopic techniques might ascertain ultrastructural features associated with better aromatic quality. In that case the better aromatic variety might be distinguished and segregated from the less desirable one. In this context, Dakshini and Heywood [3] used SEM for the study of cumin seeds, but they emphasized the need of further detailed studies in future.

The exomorphic characters are accurately studied by SEM and AFM to pin point the similarities and dissimilarities. One type of dry seed sample is collected from Gujarat (G) which is famous for cumin cultivation, and another dry seed sample was taken from West Bengal (K) where a very small quantity is produced. It is well known from earlier studies [1] that the Gujarat variety has superior aromatic qualities than the variety from West Bengal.

Samples are dried prior to marketing, so no further dehydration is needed for Scanning Electron Microscope (SEM) imaging. Samples can be directly placed on stubs and coated with gold (100 A thickness) with the gold coater. The samples were examined at 15 kV accelerating voltage with the JEOL (JEOL JSM – 5200, Tokyo, Japan) Scanning Electron Microscope. The micrographs were recorded in NOVA NP22 film.

For Atomic Force Microscope (AFM) studies, the dry seeds were fixed on 1 cm^2 glass slides. No special dehydration or coating procedures are necessary. For the best

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resolution, the hard surface of cumin seed was scanned by contact mode at room temperature [4]. The topography and phase imaging were done by an Atomic Force Microscope of Model NT-DMT SOLVER P47. AFM images

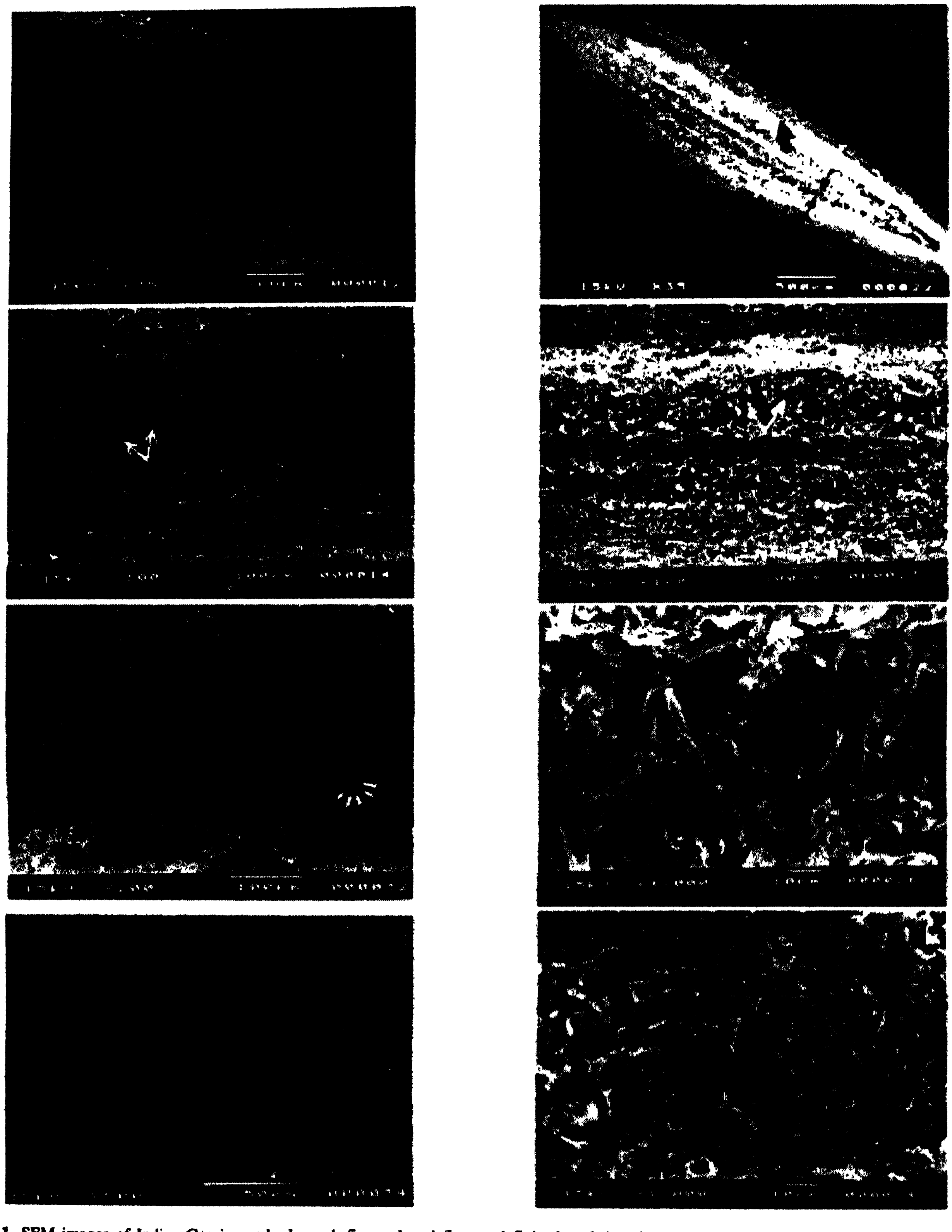

Figure 1. SEM images of Indian Cumin seeds. In each figure, bar defines a definite length in micrometer (μm).

Sample G :
(a) A cumin seed showing ridges ( )and furrows ( )
(b) papillose hairs in furrows ( ),
(c) papillose hairs showing striations ( ),
(d) assemblage of basal canals ( ) ,

Sample K :
(a) A slender cumin seed showing ridges ( ) and furrows ( ),
(b) delicate papillose hairs in furrows ( ),
(c) papillose hairs showing no striation,
(d) furrow region showing no canal.

of the seed surface were taken at scan size 350 nm × 350 nm and 3500 nm × 3500 nm. In case of the topographical image, the movement of the piezoelectric tube is vertical and the applied force is constant. In case of the phase image, the determination of the vertical movement of the piezoelectric tube is recorded by the drop in amplitude of the oscillating cantilever. The images of the structures were then measured [5] with the help of a lens with an inbuilt microscale (Graticules, UK).

SEM :

Morphologically both the cumin seeds appear ovoid elongated and laterally compressed, greyish brown in colour. At the magnification of X 35, SEM observations show that K is of a more slender form, but *G* is longer. The breadth of K and G is 0.072 ± 0.002 cm and 0.104 ± 0.005 cm respectively (Figures 1 Ga and 1 Ka).

In both the samples, ridges (costae) run longitudinally from the stylar end of the seed. In the sample G (Figure Ga) secondary ridges are strongly developed from the primary. Under the same magnification (X 35) of SEM of the sample G, ridges in between the furrows are broad (Figure Ga) while in sample K, the ridges are narrow (Figure Ka). The furrows (valleculae) appear shallow, wider and distorted (Figure Kb) against the deep, brief and sharp grooves of sample G (Figure Gb).

Papillose hairs are a characteristic feature of the seeds. It is revealed that the furrow is traversed by some stout and flexible papillose hairs at the magnification of X 100 (Figure 1 Gb). The striations are clear (Figure 1 Gc) in the papillose hairs which are long and developed from both sides of the furrows arranged in rows. The papillose hairs are profuse, slender, loose and hyphae-like (Figure 1 Kb) at the magnification X 100. They are irregularly and densely arranged with inconspicuous striations (Figure 1 Kc).

An assemblage of canals is present at the base of the upright hairs in sample G (Figure 1 Gd) at magnification X 1000. Presence of such structure is not visible (Figure1 Kd) in sample K at the magnification X 1000.

AFM :

In the topographical image under AFM of sample *G*, the surface pattern seems to be composed of some inconspicuous broad reticula which are traversed by some circular or elongated structures in bright contrast (Figure 2 Ga). In case of the phase image the undulating surface pattern appears to be composed of some interconnected stripes, which are beset with some protuberances (Figure 2 Gb). Although *G* varieties show different blister-like morphological structures, on the other hand the K varieties do not have any such definite structures as observed under AFM. The structures in K varieties are somewhat blurred and as such the measurement of size of this variety is not practicable. Therefore Table 1 shows the measurements of blister structure of G variety only (Table 1). The blister-like structures of G variety are of different sizes which are tabulated in Table 1. In each case 30 blister-like structures are measured. These are not detected in SEM micrographs and are resolved only by AFM.

Table 1. Sample G observed in AFM phase imaging showing three size classes of blister-like structures, in each case thirty structures are measured*.

	Size		Shape
	Length in nm	Breadth in nm	
1	811 ± 25	432 ± 20	Elliptical
2	324 ± 10	324 ± 10	Circular
3	108 ± 3	108 ± 3	Circular droplet

*Since the K variety under AFM does not show above such definite blister-like structures, measurements are not mentioned in the table (see text).

The colour-scale in nm shows the difference of depth in surface area of the cumin. For example, in Figure 2 (Ga), the white structures project ~200–220 nm above the surface. In all the 4 figures, the scales indicate a number of more depressed areas.

Figure 2 Ka is obtained by topographical image of sample K. The surface pattern is manifested by some areoles (black and inconspicuous brownish areas). The areoles have been merged with reticula. In the phase image (Figure 2 Kb), areoles are represented by encircled (brownish areas) or segmented structures interconnected with reticula. The overall representation is that both the areoles and reticula are distributed in more or less circular or scattered patches which are inconspicuous in topography, but prominent in the phase image. It appears that phase imaging is important to obtain more details of microstructure for additional features of the seed surface beyond SEM.

Cumin seeds have been studied thoroughly for their nutritional value [1] but adequate attention to micromorphology has not yet been paid. It is necessary to investigate whether the higher grades of aromatic variety, rich in essential oil, can be detected by screening some morphological attributes at ultrastructural and nano levels.

The papillose hairs probably protect the seed from desiccation of moisture, volatile oil and also emission of aroma and volatile oil. Moreover, the adjoining area of the

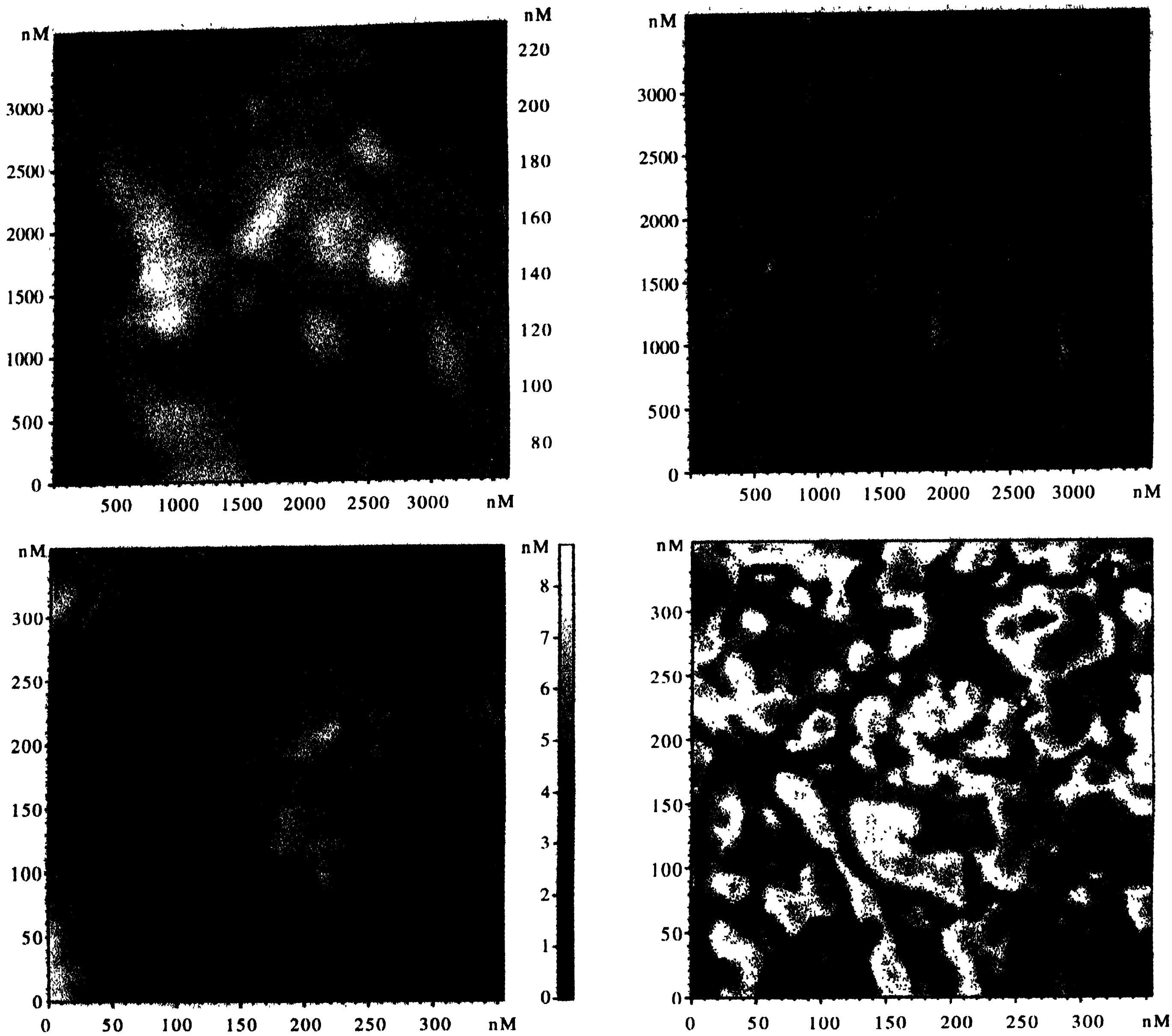

Figure 2. AFM images of Indian Cumin seeds at nanometer (nm) scale.

Sample G : (a) topography showing reticular broad (—•—), circular structure (➤), (b) phase image at scan size 3500 nm showing undulating surface (•—•) and protuberances/blister (⁚ ⁚).

Sample K : (A) topography, (b) phase image at scan size 350 nm.

furrow in sample G reveals some assemblage of canals which remain covered by some upright hairs (Figure 1 Gd). On removal of these hairs the canals become exposed at the base of the hairs. The morphological structures in the strains may also reflect the adaptation to the two eco-niches.

It is evident that the flexible papillose hairs of sample G are less susceptible to rupture and breakage than the hyphae-like hairs of sample K and they probably control the emission of aroma. The sample G represents, apparently a better economic resource, which is rather poor in case of sample K.

AFM studies show clear distinction between the two samples. At scan size 4000 X 4000 nm of sample G (Figure 2 Gb) and at 400 X 400 nm of sample K (Figure 2 Kb) interconnected reticula are present. For sample K interconnection of reticula is not located beyond 400 nm. Blister-like protuberance is also absent in sample K. It is evident from Figures 2 Gb and 2 Kb, the ultrastructures differ considerably in the phase image.

Dakshini and Heywood [3] commented that the diversity of cumin seed structure is surprisingly great and much further study will have to be undertaken to consider the genetic control, variability and adaptive significance of the features of surface ornamentation. The present approach is an important study in that direction towards undertaking future work with different strains and their aroma molecules to mark the quality of the spice.

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References

[1] J S Pruthi *Cumin seed Spices and Condiments* (New Delhi : National Book Trust of India) p114 (1998)

[2] S L Kochhar *Economic Botany in the Tropics* (2nd edn) (Delhi : Macmillan India) p604 (1998)

[3] K M M Dakshini and V H Heywoood *Biology and Chemistry of Umbelliferae* (ed) VH Heywood (London : Academic) p215 (1971)

[4] P Russell and D Batchelor *Microscopy and Analysis* **22** 13 (2001)

[5] T Guha, R Bhar, V Ganesan, A Sen and R L Brahmachary *Microscopy and Microanalysis* 7 526 (2001)